Vol. LI, No. 6 (December, 1970) was issued 22.12.70.

THE BRITISH JOURNAL OF
EXPERIMENTAL PATHOLOGY

VOL. LII FEBRUARY, 1971 NO. 1

ANTI-INSULIN SERUM, PLASMA INSULIN, AND THE HYPOGLYCAEMIA OF TOTAL PANCREATECTOMY AND PARTIAL HEPATECTOMY IN THE RAT

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Received for publication August 5, 1970

SUMMARY.—The administration of anti-insulin serum did not prevent rats from becoming hypoglycaemic following total pancreatectomy combined with partial hepatectomy, and no hyperinsulinaemia was detected. Reversing the order of sequence of the combined operation accelerated the onset of hypoglycaemia; no specific changes were found in plasma free fatty acids levels.

IN this paper we report investigations into the possible mechanism of the profound hypoglycaemia which results from the combined operation of total pancreatectomy and partial hepatectomy in the rat (Sutton and Taghizadeh, 1968). We have already shown that this is not due to glucagon deficiency (Taghizadeh and Sutton, 1969). It therefore appeared worthwhile to investigate the possibility that the hypoglycaemia was caused by the massive release of insulin, by analogy with the effects of alloxan administration. It is well recognized that, in the experimental animal, the injection of alloxan produces a triphasic blood sugar curve (Warren, Lecompte and Legg, 1966). There is first an initial rise in the blood glucose concentration, some 3 hr after administration, due to an adrenaline mediated mobilization of liver glycogen. This is followed by a phase of hypoglycaemia from 6–12 hr after injection, to be succeeded by a permanent (diabetic) hyperglycaemia at 18–24 hr. Most authorities (*e.g.* Taylor, 1968) accept that the period of hypoglycaemia is due to the massive release of insulin into the circulation from the damaged and dying β cells. The question therefore arises, could the hypoglycaemia observed after total pancreatectomy with partial hepatectomy be caused by the combination of insulin released during the blunt dissection of the diffuse rat pancreas, together with the loss of liver insulinase resulting from the partial hepatectomy?

To test this hypothesis two groups of experiments were performed. In the first, anti-insulin serum was administered to rats both before and after total pancreatectomy combined with partial hepatectomy, in order that any insulin released during the operation would be neutralized. In the second group of experiments, plasma insulin levels were estimated following the various operations, on the assumption that greater plasma concentrations of insulin would be found in the hypoglycaemic rats, if the low blood glucose levels were due to insulin released into the circulation.

As a separate study, the effect of reversing the order in which the combination of total pancreatectomy and partial hepatectomy was performed was investigated, and also estimations were made of the plasma levels of free fatty acids (FFA) under these circumstances.

MATERIALS AND METHODS

Animals.—Male white Wistar rats, weighing between 140–170 g. were used throughout the experiments.

Feeding.—The animals were fully fed on the standard laboratory rat diet (MRC 41.b food cubes) up to the time of operation, but not given food in the post-operative period (which was never longer than 8 hr). Drinking water was always freely available.

Operations.—" Total " pancreatectomy was performed by a slight modification of the technique of Scow (1957) and partial hepatectomy by the classical method of Higgins and Anderson (1931). The laparotomies involved the mobilization of the abdominal viscera (as for the other operations) but without the removal of any tissues. All these surgical techniques were carried out under open ether anaesthesia at the same time of day (between 10 a.m. and 1 p.m.). Anti-insulin serum was injected i.v. *via* the jugular vein, either at the beginning or upon completion of the operations, or both.

Collection of samples.—At the end of the experiment the animals were killed by bleeding from the abdominal aorta under ether anaesthesia and, once the blood sample for the determination of glucose had been taken, the remainder of the specimen was immediately heparinized.

Analysis.—The following estimations were performed:

(a) Blood glucose. The blood sample was used for estimation of the blood glucose by the glucose oxidase-peroxidase automated method of Faulkner (1965).

(b) Plasma FFA. Plasma from the remaining sample was separated, and FFA levels determined using an automated version of the method of Konitzer, Voigt and Solle (1964).

(c) Plasma Insulin. This was estimated by a modified double antibody method of Hales and Randle (1963) employing the Burroughs Wellcome/Radiochemical Centre kit. The Insulin Binding Reagent contained an antiserum selected for its ability to combine strongly with rat insulins. Standards were prepared from a mixture of equal weights of rat insulins I and II, and the results expressed as ng. rat insulin per ml. of plasma.

(d) Liver glycogen. Small pieces of liver, accurately weighed, of about 200 mg., were placed in 2 ml. of 30 per cent (w/v) potassium hydroxide solution prior to estimation of liver glycogen by the method of Seifter, Dayton, Novic and Muntwyler (1950).

Anti-insulin serum.—AIS was obtained from guinea-pigs immunized with repeated doses of beef insulin in adjuvant.

RESULTS

Experiments with AIS

The effect of AIS on normal rats.—It was first necessary to show that the preparation of AIS actually used was capable of inactivating rat insulin *in vivo*; this is shown in the first table in which a satisfactory, temporary hyperglycaemia followed its administration.

TABLE I.—*Blood Glucose in mg./100 ml. (Mean of 2 Results) in 8 Rats Given 0·2 ml. AIS by i.v. Injection*

No. of rats	Time after AIS	Blood glucose
2	0	82
2	30 min.	209
2	60 min.	257
2	180 min.	96

The effect of AIS on the hypoglycaemia following total pancreatectomy combined with partial hepatectomy.—In these experiments the AIS was given firstly immediately after the combined operation, and then (in a second group of animals) both immediately before and after operation. Table II gives the results and shows that the administration of AIS did not influence the development of hypoglycaemia.

TABLE II.—*Mean Blood Glucose in mg./100 ml. ± Standard Error in Rats 6 hr After Total Pancreatectomy with Partial Hepatectomy*

0·2 ml. AIS post-op.	0·2 ml. AIS pre-op. +0·2 ml. AIS post-op.	No AIS
20·5±5·5 (8)	18·7±9·0 (6)	22·0±7·9 (6)

Total numbers of animals are shown in parentheses.

Plasma insulin levels

In this group of experiments plasma insulin levels were estimated in rats undergoing either the combination of pancreatectomy with partial hepatectomy, or partial hepatectomy alone, or laparotomy. It was at this stage that an unexpected difficulty arose in that not all of the rats subjected to the combined operation became hypoglycaemic. The possible reasons for this complication are elaborated further (in the Discussion) but whatever the explanation, only about one half of the animals developed the profound lowering of the blood glucose characteristic of this experimental situation. For this reason, in Table III, the rats subjected to the combined operation are separated into 2 groups on the basis of whether or not hypoglycaemia was present.

TABLE III.—*Mean Blood Glucose (mg./100 ml.), Plasma Insulin (ng./ml.) ± Standard Error, in Rats at Various Times After Operation*

Operation	Pancreatectomy with partial hepatectomy —hypoglycaemic	Pancreatectomy with partial hepatectomy —non-hypoglycaemic	Partial hepatectomy	Laparotomy
No. of rats	11	13	6	5
Blood glucose	10·5±2·0	67·3±9·6	58·2±1·9	95±8·5
Plasma insulin	2·2±0·2	1·4±0·2	6·4±1·3	6·8±1·3
Mean time of death after operation, hr.	3·8 (range 2–7)	6·0 (range 4–8)	6·0 (killed)	6·0 (killed)

It can be seen that no hyperinsulinaemia was found in rats subjected to pancreatectomy combined with partial hepatectomy, the mean plasma insulin levels being much lower than that found in rats undergoing partial hepatectomy or laparotomy.

The effect of reversing the sequence of the combined operation

In an attempt to decrease the delay between the operations and the onset of hypoglycaemia, the normal sequence of the combined operation was reversed. A comparison was made between total pancreatectomy followed by partial hepatectomy, and partial hepatectomy followed by total pancreatectomy. Table IV shows that performing the partial hepatectomy first, accelerated the onset of hypoglycaemia. In both groups, however, only a trace of hepatic glycogen remained at the time of death.

TABLE IV.—*Time (hr After Operation) of Fatal Hypoglycaemia (Blood Glucose < 20 mg./100 ml.) with Hepatic Glycogen (per cent)*

Operation	Pancreatectomy followed by partial hepatectomy	Partial hepatectomy followed by pancreatectomy
No. of rats	6	6
Mean time (hr)	7·0 (range 6–8)	2·5 (range 2–3)
Liver glycogen	0·3 (range 0·2–0·4)	0·2 (range 0·1–0·4)

Plasma levels of FFA

Finally, the plasma FFA levels were estimated as shown in Table V.

TABLE V.—*Mean Plasma FFA (mEq./l.) ± Standard Error, in Rats 6 hr After Operation*

Operation	Pancreatectomy with partial hepatectomy	Partial hepatectomy	Laparotomy
Plasma FFA	1·30±0·08 (9)	1·48±0·09 (6)	0·64±0·05 (5)

The numbers of animals are shown in parentheses.

DISCUSSION

The effect of anti-insulin serum

It is clear from Table II that the administration of anti-insulin serum, given both immediately before and after the operation, did nothing to prevent (or even delay) the hypoglycaemia following the combination of total pancreatectomy with partial hepatectomy. The results in Table I, where the i.v. injection of anti-insulin serum into normal animals resulted in hyperglycaemia within 30 min., show that the serum used was capable of neutralizing rat insulin in the dosage given. This is therefore evidence against the idea that the hypoglycaemia produced by these operations is due to insulin released by the pancreas during its resection.

The levels of plasma insulin

In the rabbit Howell and Taylor (1967) showed that the serum insulin content rose during the hypoglycaemic phase of alloxan administration, evidence that the lowering of the blood glucose in this situation is caused by insulin released by the damaged β cells of the pancreatic islets. However, our results (Table III) show that the levels of plasma insulin were always much lower in the rats which had undergone total pancreatectomy combined with partial hepatectomy (regardless

of whether hypoglycaemia developed or not) than in the controls subjected to either laparotomy, or partial hepatectomy, alone.

Taken together, both the failure of anti-insulin serum to maintain a normal level of blood glucose and the absence of any rise in plasma insulin levels following total pancreatectomy combined with partial hepatectomy would seem to disprove the hypothesis that endogenous insulin release is the cause of the hypoglycaemia. In any case, on purely theoretical grounds, the action of alloxan may be regarded as a somewhat special case, since the drug selectively destroys the β cells, but spares both the rest of the islet tissue and also the exocrine pancreas. By contrast, during the operation of pancreatectomy, the technique of blunt dissection must cause considerable damage to the organ as a whole, thereby releasing pancreatic enzymes which might inactivate any free insulin.

The sequence of the operations

As can be seen in Table III, one of the problems encountered during these experiments has been the failure of some rats to become hypoglycaemic following total pancreatectomy combined with partial hepatectomy. There are two possible reasons for this.

(a) In many cases the actual timing of the onset of hypoglycaemia is probably the critical factor. In these present experiments all the rats were killed by 8 hr after operation, but had the experiments been allowed to continue longer it is possible that hypoglycaemia would have developed. We have previously recorded (Taghizadeh and Sutton, 1969) that this can occur as late as 9 hr post-operatively, and we have occasionally observed animals in which a blood glucose of less than 10 mg. per cent was associated with death as long as 29 hr later. In an attempt to decrease the variability of the timing, the order in which the operation was performed was reversed. In all our previous work the pancreatectomy part of the combined operation had been carried out before the partial hepatectomy. Table IV shows the effect of reversing this order and performing the partial hepatectomy first. This decreased the time interval between the completion of the combined operation and the onset of signs of hypoglycaemia. This effect is probably related to the question of the hepatic glycogen reserves, since we have already shown (Sutton and Taghizadeh, 1968) that the fall in blood glucose occurs only when the liver's stores of glycogen have been depleted (*i.e.* that gluconeogenesis but not glycogenolysis is defective). The removal of two-thirds of the liver first, followed by the much lengthier operation of total pancreatectomy, must exhaust the glycogen reserves quicker. This is also shown in Table IV where all of the hypoglycaemic rats were found to have only traces of glycogen in their livers at the time of death.

(b) In some instances the animals died in coma within 8 hr of the combined operation and yet proved to have normal blood glucose levels. At autopsy no technical or anatomical cause of death (*e.g.* haemorrhage or intestinal infarction) could be found, thereby raising the suspicion that some other metabolic effect was the fatal event. Experiments are continuing in an investigation of this possibility, concentrating in particular upon the levels of blood lactate, which might be expected to be raised if gluconeogenesis is defective.

FFA levels

Since both glucose and FFA are the principal transport forms of " fuel " in

the mammalian body (Himsworth, 1968) it was of some interest to determine the levels of plasma FFA in these experiments. It can be seen from Table V that there was no significant difference in these levels between animals undergoing the combination of pancreatectomy with partial hepatectomy, and those subjected to partial hepatectomy alone (though both groups had higher plasma FFA concentrations than laparotomized rats). Nor, within the group of animals undergoing the combined operation, was there any relationship between the plasma levels of glucose and FFA (*i.e.* the most severely hypoglycaemic rats still had FFA levels within the normal range).

We wish to thank Professor J. F. Smith for his helpful advice, and Dr. Keith Taylor at the University of Sussex for the rat insulins. This work was aided by an MRC grant (P.M.S.) for which grateful acknowledgement is made.

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